```
     (FILE 'USPAT' ENTERED AT 19:18:27 ON 15 SEP 1999)
L1          159 S CD33
L2           28 S L1(P)LEUKEMIA
L3           20 S L2(P)(ANTIBOD?)
L4            6 S L3(P)(PHARMA? OR CLINIC? OR THERAP?)
L5           22 S IMMUNOCONJUGATE#(P)GELONIN
L6           45 S L1(P)(PURGE## OR BONE(W)MARROW)
L7           44 S L6 NOT L4
L8           42 S L7(P)ANTIBOD?
L9           28 S L6(P)ANTIBOD?
L10          27 S L9 NOT L4
L11           1 S L9(P)PURGE###
L12         255 S BONE(W)MARROW(P)(FREEZ? OR FROZEN)
                E ROSENBLUM, MICHAEL?/IN
L13           4 S E1,E2
```

L5: 4 of 22

TITLE: Polynucleotides encoding gelonin sequences
US PAT NO: 5,837,491 [IMAGE AVAILABLE] DATE ISSUED: Nov. 17, 1998
APPL-NO: 08/646,360 DATE FILED: May 13, 1996
PCT-NO: PCT/US94/05348 PCT-FILED: May 12, 1994
371-DATE: May 13, 1996
102(E)-DATE: May 13, 1996
PCT-PUB-NO: WO94/26910 PCT-PUB-DATE: Nov. 24, 1994
REL-US-DATA: Continuation-in-part of Ser. No. 64,691, May 12, 1993, abandoned, which is a continuation-in-part of Ser. No. 988,430, Dec. 9, 1992, Pat. No. 5,416,202, which is a continuation-in-part of Ser. No. 901,707, Jun. 19, 1992, Pat. No. 5,376,546, which is a continuation-in-part of Ser. No. 787,567, Nov. 4, 1991, abandoned.

L5: 9 of 22

TITLE: Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype
US PAT NO: 5,686,578 [IMAGE AVAILABLE] DATE ISSUED: Nov. 11, 1997
APPL-NO: 08/286,430 DATE FILED: Aug. 5, 1994

L5: 10 of 22

TITLE: Anti-AIDS immunotoxins
US PAT NO: 5,645,836 [IMAGE AVAILABLE] DATE ISSUED: Jul. 8, 1997
APPL-NO: 08/422,578 DATE FILED: Apr. 14, 1995

L5: 14 of 22

TITLE: Preparation and use of immunoconjugates
US PAT NO: 5,443,953 [IMAGE AVAILABLE] DATE ISSUED: Aug. 22, 1995
APPL-NO: 08/162,912 DATE FILED: Dec. 8, 1993

L5: 15 of 22

TITLE: Materials comprising and methods of preparation and use for ribosome-inactivating proteins
US PAT NO: 5,416,202 [IMAGE AVAILABLE] DATE ISSUED: May 16, 1995
APPL-NO: 07/988,430 DATE FILED: Dec. 9, 1992
REL-US-DATA: Continuation-in-part of Ser. No. 901,707, Jun. 19, 1992, which is a continuation-in-part of Ser. No. 787,567, Nov. 4, 1991, abandoned.

US PAT NO: 5,730,982 [IMAGE AVAILABLE] L11: 1 of 1

SUMMARY:

BSUM(3)

Mouse monoclonal **antibody** M195 is an IgG2a developed at Sloan-Kettering Institute (Tanimoto M., Scheinberg D A, Cordon-Cardo C, et al. Leukemia 3:339-348, 1989.. . . but not to the earliest myeloid progenitors. The target antigen is not expressed on any other hematopoietic or non-hematopoietic tissue. **Antibodies** to a related antigen on the same protein (**CD33**), My9 and L4F3, are currently being used to **purge bone marrow** of ANLL before autologous transfusion (Bernstein I D, Singer J W, Andrews R G, et al. J Clin Invest 79:1153-1159,. . . able to kill leukemia cells with rabbit or guinea pig complement, but not by use of human complement or human **antibody**-dependent cellular cytotoxicity in vitro. Activation of t

M195 is a murine IgG2a monoclonal **antibody** that binds **CD33** antigen and has **therapeutic** potential for the treatment of myloid **leukemia** (Tanimoto et al., **Leukemia** 3:339 (1989) and Scheinberg et al., **Leukemia** 3:440 (1989)). M195 binds to early myeloid progenitor cells, some monocytes, and the cells of most myeloid

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SYSTEM:OS  - DIALOG OneSearch
  File 149:TGG Health&Wellness DB(SM)  1976-1999/Sep W1
        (c) 1999 The Gale Group
  File   5:Biosis Previews(R)  1969-1999/Aug W2
        (c) 1999 BIOSIS
  File  73:EMBASE  1974-1999/Aug W3
        (c) 1999 Elsevier Science B.V.
  File 156:Toxline(R)  1965-1999/Jul
        (c) format only 1999 The Dialog Corporation
  File 159:Cancerlit  1975-1999/Aug
        (c) format only 1999 Dialog Corporation
*File 159: reloaded, note accession numbers changed.
  File 155:MEDLINE(R)  1966-1999/Nov W1
        (c) format only 1999 Dialog Corporation
*File 155: reloaded, note accession numbers changed.
  File 434:SciSearch(R) Cited Ref Sci  1974-1989/Dec
        (c) 1998 Inst for Sci Info
  File  76:Life Sciences Collection  1982-1999/Jul
        (c) 1999 Cambridge Sci Abs
  File 652:US Patents Fulltext  1971-1979
        (c) format only 1999 The Dialog Corp.
*File 652: Reassignment data now current through 07/09/99
Reexamination, extension, expiration, reinstatement updated weekly.
  File 653:US Pat.Fulltext  1980-1989
        (c) format only 1999 Knight-Ridder Info
*File 653: Reassignment data now current through 07/09/99.
Reexamination, extension, expiration, reinstatement updated weekly.
  File 654:US Pat.Full.  1990-1999/Sep 07
        (c) format only 1999 The Dialog Corp.
*File 654: Reassignment data current through 07/09/99.
  File 353:APIPAT  1964-1999/Sep W2
        (c) 1999 Elsevier Engineering Info., Inc.
*File 353: !!Abstracts now display in formats AB,7,5,9,29,49!!  Output
prices are unchanged.  Usage is limited to 2hrs./yr.  See HELP NEWS 353
  File 357:Derwent Biotechnology Abs  1982-1999/Aug B1
        (c) 1999 Derwent Publ Ltd
*File 357: Derwent changes DialUnit pricing from May 1, 1999.  See
HELP DERWENT for details.

      Set  Items  Description
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?
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Set	Items	Description
S1	3886	CD33
S2	4284	L1(5N)LEUKEMIA
S3	4	S2(5N)(IMMUNOCONJUGATE? OR IMMUNOTHERAP?)
S4	2	RD (unique items)
S5	404	S1(5N)ANTIBOD?
S6	30	S5(5N)(THERAP? OR CLINIC? OR PHARMA?)
S7	14	RD (unique items)
S8	8	S5(5N)(TOXIN OR GELONIN)
S9	5	RD (unique items)
S10	268	S2(5N)(BONE(W)MARROW)
S11	0	S10(5N)PURGE?
S12	73	S10/1980:1992
S13	35	RD (unique items)
S14	1235	GELONIN
S15	46	S14(5N)IMMUNOCONJUGATE
S16	20	RD (unique items)
S17	423	(BONE(W)MARROW)(5N)(FREEZ?)
S18	0	S17(5N)TRANSFER
S19	180	S17/1980:1992
S20	126	RD (unique items)

9/7/4 (Item 2 from file: 159)
DIALOG(R)File 159:Cancerlit

01236604 96710053 ICDB/96710053
Humanized M195 monoclonal antibody conjugated to recombinant gelonin has antileukemic activity (Meeting abstract).
Pagliaro LC; Liu B; Munker R; Andreeff M; Freireich EJ; Scheinberg DA; Rosenblum MG
UT MD Anderson Cancer Center, Houston, TX 77030
Proc Annu Meet Am Soc Clin Oncol; 15:A1047 1996 ISSN 0732-183X
Languages: ENGLISH
Document Type: MEETING ABSTRACTS
The recently characterized immunotoxin HuM195-**gelonin** consists of a humanized anti-**CD33** monoclonal **antibody** conjugated to the single-chain plant toxin gelonin. Binding of the immunotoxin to hematopoietic cells which express the CD33 differentiation antigen has been demonstrated and results in cytotoxicity due to ribosomal inactivation by gelonin. Blast cells from most patients with acute myelogenous leukemia express CD33, whereas normal stem cells necessary for maintenance of hematopoiesis do not. We asked whether an immunoconjugate using recombinant gelonin rather than plant gelonin is toxic to AML cell lines and primary AML blasts obtained from patients and exposed to the immunotoxin in vitro. The CD33-positive cell lines HL60, OCI/AML2, and OCI/AML5 showed decreased proliferation when exposed to immunotoxin for 24 to 72 hr. The CD33-negative cell line OCI/AML3 was relatively resistant to HuM195, and all cell lines were resistant to equimolar concentrations of unconjugated antibody and gelonin. Primary blast cultures from 7 patients with AML had CD33 detectable on 75.7% to 99.8% of cells by flow cytometry, and all showed antibody-mediated decreases in clonogenic cell survival during 24 hr incubation with the immunotoxin. There was a trend toward lower IC50 for immunotoxin in samples with higher CD33 expression ($r = -0.25$, $p = 0.59$). Cells selected for low CD33 expression by cell sorting or by prolonged incubation with immunotoxin could re-express CD33 at baseline levels and remained sensitive to immunotoxin. We conclude that humanized M195 conjugated to recombinant gelonin has antileukemic activity and should be considered for clinical testing in Phase I trials. (C) American Society of Clinical Oncology 1997

9/7/5 (Item 1 from file: 76)
DIALOG(R)File 76:Life Sciences Collection

02035242 3909117
Antileukemic activity of recombinant humanized M195-gelonin immunotoxin in nude mice
Xu, Y.; Xu, Q.; Rosenblum, M.G.; Scheinberg, D.A.
Memorial Sloan-Kettering Cancer Cent., 1275 York Ave., New York, NY 10021, USA
LEUKEMIA vol. 10, no. 2, pp. 321-326 (1996)
ISSN: 0887-6924
DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH
SUBFILE: Immunology Abstracts

A leukemia-selective immunotoxin was constructed by linking recombinant gelonin (rGel), a single chain ribosome inhibitory protein, to recombinant humanized M195 antibody (HuM195), which recognizes the cell-surface protein designated CD33. CD33 is an antigen found on myeloid leukemia blasts as

well as myeloid progenitor cells but it is not expressed in detectable amounts on the ultimate hematopoietic progenitor stem cell. Our previous studies indicated that a non-recombinant humanized immunotoxin displayed specific, potent toxicity towards CD33-positive cells but not to CD33-negative cells in vitro. In the current study, a recombinant humanized immunotoxin, HuM195-rGel, was evaluated in vivo in a nude mouse model of human myeloid leukemias. HuM195-rGel was found to target leukemia cells rapidly in vivo and was subsequently internalized into the cells. For trials in vivo, nude mice were injected (ip) with 10 super(7) log-phase HL60 human leukemia cells 10 days prior to the start of i.p. HuM195-rGel treatments. HuM195-rGel demonstrated significant tumor suppressive activity in this model. While all mice treated with either saline, rGel alone, or HuM195 plus unconjugated rGel (at 10 or 14 days after transplantation) had rapid tumor growth or early deaths, 50% of mice treated with HuM195-rGel failed to develop leukemic tumors for 5 months and the other 50% had significantly retarded tumor growth after treatment with HuM195-rGel. Mice treated at later times (28 days after transplantation of leukemia cells) also showed delayed leukemia cell growth, but no cures. These data show that HuM195-rGel can target leukemia cells in vivo and can result in

16/7/8 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05741182 BIOSIS NO.: 000084089589
THE ANTILEUKEMIC EFFICACY OF AN IMMUNOTOXIN COMPOSED OF A MONOCLONAL ANTI-THY-1 ANTIBODY DISULFIDE LINKED TO THE RIBOSOME-INACTIVATING PROTEIN GELONIN

AUTHOR: SCOTT C F JR; GOLDMACHER V S; LAMBERT J M; CHARI R V J; BOLENDER S; GAUTHIER M N; BLATTLER W A
AUTHOR ADDRESS: DIV. TUMOR IMMUNOL., DANA-FARBER CANCER INST., 44 BINNEY ST., BOSTON, MASS. 02115.

JOURNAL: CANCER IMMUNOL IMMUNOTHER 25 (1). 1987. 31-40.
FULL JOURNAL NAME: Cancer Immunology Immunotherapy
CODEN: CIIMD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: We prepared an immunoconjugate consisting of a monoclonal antibody recognizing the Thy-1 antigen and the ribosome-inactivating protein gelonin linked by a disulfide bond. This immunotoxin preparation was judged to contain less than 5% free antibody or gelonin. It was highly toxic in vitro in an antigen-specific fashion to the Thy-1 expressing RADA leukemia of A/J mice. The IC50 of this preparation on RADA in vitro was 10-12 M, while the IC50 on the Thy-1 negative S1509a fibrosarcoma of A/J mice was 10-7 M. The toxicity of this immunoconjugate was also measured in a direct proliferation assay and it was found that a 4-h exposure and a 24-h exposure of RADA cells to a 1 nM concentration of immunotoxin killed 90% and 99.9% of cells, respectively. Furthermore, efficacy in vitro was not due to the intrinsic susceptibility of RADA cells to this type of immunotoxin, as one prepared with gelonin and an antibody recognizing the TLa determinant on this leukemia had no efficacy in vitro. Clearance of the anti-Thy-1-**gelonin immunoconjugate** from the circulation of A/J mice after i.v. injection was rapid, especially during the first 8 h after injection, possibly because of binding to Thy-1 expressing tissue. Delivery of immunoconjugate to ascitic tumor in vivo was substantially better if the immunoconjugate was given by i.p. injection, rather than by the i.v. route. When given either i.v. or i.p. at the time of i.p. tumor inoculation in vivo, the anti-Thy-1-gelonin immunotoxin showed potency in an antigen-specific fashion; while this immunoconjugate prolonged survivor and frequently cured RADA-inoculated mice, neither anti-Thy-1-antibody, gelonin, a combination of the two, nor immunotoxin of irrelevant specificity had any significant effect on survival. Anti-Thy-1-gelonin also had no effect on survival of A/J mice inoculated i.p. with S1509a. Furthermore, it was determined that a single i.p. dose of anti-Thy-1-gelonin killed 90% to 99% cells in vivo, and that the immunoconjugate was about as effective in this model as either adriamycin or cytoxan.

16/7/9 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05699886 BIOSIS NO.: 000084048291
IMMUNOTOXINS TO A HUMAN MELANOMA-ASSOCIATED ANTIGEN COMPARISON OF GELONIN

WITH RICIN AND OTH[redacted]A CHAIN CONJUGATES

AUTHOR: SIVAM G; PEARSON J W; BOHN W; OLDHAM R K; SADOFF J C; MORGAN A C JR
AUTHOR ADDRESS: NEORX CORPORATION, 410 WEST HARRISON STREET, SEATTLE, WASHINGTON 98119.

JOURNAL: CANCER RES 47 (12). 1987. 3169-3173.
FULL JOURNAL NAME: Cancer Research
CODEN: CNREA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Gelonin, a ribosome-inactivating protein from the seeds of Gelonium multiflorum, has been conjugated to antibodies. Previous reports have indicated variable potency of such immunotoxins. The lack of toxicity of **gelonin**, however, makes it attractive for **immunoconjugate** production. The ribosome-inactivating protein was covalently linked (using N-succinimidyl-3-(2-pyridyldithio)propionate) to monoclonal antibody, 9.2.27, directed to a human melanoma-associated glycoprotein/proteoglycan. The immunoconjugate showed high selectivity with dose-dependent cytotoxic activity to cultured human melanoma cells (50% inhibitory dose; 1-3 .times. 10-11 M versus antigen-positive cells; 1-3 .times. 10-7 M versus antigen-negative cells). Specificity and immunoreactivity of the conjugate were similar to those of unconjugated antibody. Biodistribution studies with iodine trace-labeled conjugate in nude mice indicated that tumor localization of the gelonin conjugate was decreased compared to unconjugated antibody. However, a significant therapeutic effect of the conjugate was found with multiple but not single dose i.v. treatment in nude mice bearing established palpable melanoma. These in vivo experiments showed that gelonin conjugates (a) are not toxic up to 2 mg total dose/mouse and (b) significantly retarded the growth of established s.c. tumor. Comparison of gelonin conjugates in vitro and in vivo with other A-chain conjugates of 9.2.27 (abrin and ricin) indicated that gelonin had similar potency, better selectivity, better tumor localization, and more significant therapeutic effects.

16/7/12 (Item 1 from file: 159)
DIALOG(R)File 159:Cancerlit

01236604 96710053 ICDB/96710053

Humanized M195 monoclonal antibody conjugated to recombinant gelonin has antileukemic activity (Meeting abstract).

Pagliaro LC; Liu B; Munker R; Andreeff M; Freireich EJ; Scheinberg DA; Rosenblum MG

UT MD Anderson Cancer Center, Houston, TX 77030

Proc Annu Meet Am Soc Clin Oncol; 15:A1047 1996 ISSN 0732-183X

Languages: ENGLISH

Document Type: MEETING ABSTRACTS

The recently characterized immunotoxin HuM195-gelonin consists of a humanized anti-CD33 monoclonal antibody conjugated to the single-chain plant toxin gelonin. Binding of the immunotoxin to hematopoietic cells which express the CD33 differentiation antigen has been demonstrated and results in cytotoxicity due to ribosomal inactivation by gelonin. Blast cells from most patients with acute myelogenous leukemia express CD33, whereas normal stem cells necessary for maintenance of hematopoiesis do not. We asked whether an **immunoconjugate** using recombinant **gelonin** rather than plant gelonin is toxic to AML cell lines and primary AML blasts obtained from patients and exposed to the immunotoxin in vitro. The CD33-positive cell lines HL60, OCI/AML2, and OCI/AML5 showed decreased proliferation when exposed to immunotoxin for 24 to 72 hr. The CD33-negative cell line OCI/AML3 was relatively resistant to HuM195, and all cell lines were resistant to equimolar concentrations of unconjugated antibody and gelonin. Primary blast cultures from 7 patients with AML had CD33 detectable on 75.7% to 99.8% of cells by flow cytometry, and all showed antibody-mediated decreases in clonogenic cell survival during 24 hr incubation with the immunotoxin. There was a trend toward lower IC50 for immunotoxin in samples with higher CD33 expression ($r = -0.25$, $p = 0.59$). Cells selected for low CD33 expression by cell sorting or by prolonged incubation with immunotoxin could re-express CD33 at baseline levels and remained sensitive to immunotoxin. We conclude that humanized M195 conjugated to recombinant gelonin has antileukemic activity and should be considered for clinical testing in Phase I trials. (C) American Society of Clinical Oncology 1997

20/3/10 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07267706 BIOSIS NO.: 000090047583
A SIMPLIFIED METHOD FOR PURIFICATION CONCENTRATION AND **FREEZING** OF **BONE MARROW** CELLS FOR AUTOLOGOUS TRANSPLANTATION

AUTHOR: WENDEL S; DAYAWANSA T; DAS P C; SMIT SIBINGA C T
AUTHOR ADDRESS: RED CROSS BLOOD BANK GRONINGEN-DRENTHE, PROF RANKESTRAAT 42-44, 9713 GG GRONINGEN, NETHERLANDS.

JOURNAL: INT J ARTIF ORGANS 13 (4). 1990. 247-254.
FULL JOURNAL NAME: International Journal of Artificial Organs
CODEN: IJAOD
RECORD TYPE: Abstract

21/3/3 (Item 3 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01297906 SUPPLIER NUMBER: 10764256 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Therapeutic effects of genetically engineered toxin (DAB486 IL-2) in patient with chronic lymphocytic leukemia.
LeMaistre, Charles F.; **Rosenblum, Michael G.**; Reuben, James M.; Parkinson, David R.; Meneghetti, Carole M.; Parker, Karen; Shaw, Jill P.; Deisseroth, Albert B.; Woodworth, Thasia
The Lancet, v337, n8750, p1124(2)
May 11,
1991
PUBLICATION FORMAT: Magazine/Journal ISSN: 0099-5355 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Professional

7/7/9 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE

04613127 EMBASE No: 1991107170
A phase I trial of monoclonal antibody M195 in acute myelogenous leukemia: Specific bone marrow targeting and internalization of radionuclide

Scheinberg D.A.; Lovett D.; Divgi C.R.; Graham M.C.; Berman E.; Pentlow K.; Feirt N.; Finn R.D.; Clarkson B.D.; Gee T.S.; Larson S.M.; Oettgen H.F.; Old L.J.
Mem. S.-Kettering Cancer Ctr., 1275 York Ave,New York, NY 10021 United States

Journal of Clinical Oncology (J. CLIN. ONCOL.) (United States) 1991, 9/3 (478-490)

CODEN: JCOND ISSN: 0732-183X
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Ten patients with myeloid leukemias were treated in a phase I trial with escalating doses of mouse monoclonal antibody (mAb) M195, reactive with CD33, a glycoprotein found on myeloid leukemia blasts and early hematopoietic progenitor cells but not on normal stem cells. M195 was trace-labeled with iodine-131 (sup 1sup 3sup 1I) to allow detailed pharmacokinetic and dosimetric studies by serial sampling of blood and bone marrow and whole-body gamma-camera imaging. Total doses up to 76 mg were administered safely without immediate adverse effects. Absorption of M195 onto targets in vivo was demonstrated by biopsy, pharmacology, flow cytometry, and imaging; saturation of available sites occurred at doses >= 5 mg/msup 2. The entire bone marrow was specifically and clearly imaged beginning within hours after injection; optimal imaging occurred at the lowest dose. Bone marrow biopsies demonstrated significant dose-related uptake of M195 as early as 1 hour after infusion in all patients, with the majority of the dose found in the marrow. Tumor regressions were not observed. An estimated 0.33 to 1.0 rad/mCi sup 1sup 3sup 1I was delivered to the whole body, 1.1 to 6.1 rad/mCi was delivered to the plasma, and up to 34 rad/mCi was delivered to the plasma, and up to 34 rad/mCi was delivered to the red marrow compartment. sup 1sup 3sup 1I-M195 was rapidly modulated, with a majority of the bound immunoglobulin G (IgG) being internalized into target cells in vivo. These data indicate that whole bone marrow ablative doses of sup 1sup 3sup 1I-M195 can be expected. The rapid, specific, and quantitative delivery to the bone marrow and the efficient internalization of M195 into target cells in vivo also suggest that the delivery of other isotopes such as auger or alpha emitters, toxins, or other biologically important molecules into either leukemia cells or normal hematopoietic progenitor cells may be feasible.